Science of the Total Environment 639 (2018) 360–366

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Boosted nutritional quality of food by CO_2 enrichment fails to offset energy demand of herbivores under ocean warming, causing energy depletion and mortality

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HIGHLIGHTS

- We tested if energy transfer via feeding is boosted under future seawater conditions.
- Energy budget of herbivores and nutritional quality of their food were determined.
- Energy budget raised by feeding on the more nutritious food under ocean acidification.
- When combined with warming, however, mass mortality occurred due to energy depletion.
- Enhanced food quality inadequately offsets the energy demand under ocean warming.

GRAPHICAL ABSTRACT

Ocean acidification and warming
Impact the physiology of herbivores
Increase the nutritional quality of primary producers
Elevate the energy demand for maintenance
Boost the energy gain of herbivores
Reduce the fitness and survival of herbivores

ARTICLE INFO

Article history:
Received 23 March 2018
Received in revised form 5 May 2018
Accepted 13 May 2018
Available online 20 May 2018

Editor: Henner Hollert

Keywords:
Fitness and survival
Gastropod
Herbivory
Ocean acidification
Ocean warming
Trophic transfer

ABSTRACT

The CO_2-boosted trophic transfer from primary producers to herbivores has been increasingly discovered at natural CO_2 vents and in laboratory experiments. Despite the emerging knowledge of this boosting effect, we do not know the extent to which it may be enhanced or dampened by ocean warming. We investigated whether ocean acidification and warming enhance the nutritional quality (C:N ratio) and energy content of turf algae, which is speculated to drive higher feeding rate, greater energy budget and eventually faster growth of herbivores. This proposal was tested by observing the physiological (feeding rate, respiration rate and energy budget) and demographic responses (growth and survival) of a common grazing gastropod (*Phasianella australis*) to ocean acidification and warming in a 6-month mesocosm experiment. Whilst we observed the boosting effect of ocean acidification and warming in isolation on the energy budget of herbivores by either increasing feeding rate on the more nutritious algae or increasing energy gain per feeding effort, their growth and survival were reduced by the sublethal thermal stress under ocean warming, especially when both climate change stressors were combined. This reduced growth and survival occurred as a consequence of depleted energy reserves, suggesting that the boosting effect via trophic transfer might not sufficiently compensate for the increased energy demand imposed by ocean warming. In circumstances where ocean acidification and warming create an energy demand on herbivores that outweighs the energy enhancement of their food (i.e. primary producers), the performance of herbivores to control their blooming resources likely deteriorates and thus runaway primary production ensues.

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https://doi.org/10.1016/j.scitotenv.2018.05.161
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1. Introduction

Given the global acceleration in anthropogenic carbon dioxide emission, marine organisms and the ecosystems that they support are considered to be threatened by ocean acidification and warming, possibly altering the functioning of marine ecosystems in the near future (Fabry et al., 2008; Nagelkerken and Connell, 2015). However, recent evidence shows that trophic dynamics in a community may allow marine organisms and their communities to resist the impacts of future climate (Goldenberg et al., 2017, 2018). Indeed, the strength of consumer-resource interactions is often critical to shaping the stability of food webs (May, 1973; McCann et al., 1998), particularly where they adjust to stabilize ecosystem processes during environmental changes (Connell and Ghedini, 2015). Herbivory is a key class of consumer-resource interaction because it determines the flux of energy and materials from primary producers to herbivores and has the capacity to compensate for climate-driven changes in primary production (Ghedini et al., 2015).

Recent studies reveal that the links between primary production and its consumption can be facilitated by ocean acidification which boosts the trophic transfer of carbon from primary producers to herbivores (Goldenberg et al., 2017), so that the reproductive output (Heldt et al., 2016) and population size of herbivores can be promoted (Connell et al., 2017). Indeed, CO_2 can act as a resource for primary producers and hence propagate throughout food webs, including omnivores and carnivores (Vizzini et al., 2017). Key to understanding such community-wide changes may centre on recognising how the nutritional quality of primary producers might be boosted by CO_2 enrichment (Falkenberg et al., 2013, but see Gordillo et al., 2016), possibly leading to ensuing faster feeding rate, greater energy gain and eventually better fitness of herbivores (Cruz-Rivera and Hay, 2001; Hemmi and Jormalainen, 2002; Ghedini and Connell, 2016; Kamya et al., 2017). In this regard, the energetic costs of ocean acidification (e.g. acid-base regulation and kinetic constraints on calcification; Pörtner, 2008; Thomsen et al., 2015), may be offset. Such indirect effect of CO_2 (i.e. boosted nutritional quality of food) may be a key mechanism that allows herbivores to maintain their fitness and survival under ocean acidification.

Warming may also enhance the trophic transfer of energy from primary producers to herbivores via two possible mechanisms. First, warming may increase the nutritional quality of primary producers (Renaud et al., 2002; Gordillo et al., 2016), and hence indirectly promotes the energy gain of herbivores via consumption. Second, warming can elevate the metabolic rate of herbivores, which in turn leads to greater feeding rate and ultimately energy gain (Brown et al., 2004). Critically, however, such positive influence of warming on consumption depends on the thermal tolerance of herbivores themselves. Where the temperature exceeds their thermal tolerance, the energy supply from aerobic metabolism falls below the energy demand for basal maintenance (i.e. energy deficit, Pörtner, 2002, 2012; Sokolova, 2013). As a result, the physiological and behavioural performance of herbivores deteriorates, leading to reduced fitness and survival (Sokolova, 2013; Leung et al., 2017a). Therefore, the key is whether the boosted energy transfer from primary producers to herbivores can sufficiently compensate for the elevated energy demand under future climatic conditions.

In this study, we assessed whether ocean acidification and warming enhance the energy transfer from primary producers to herbivores, which can in turn compensate for the additional energetic costs imposed on herbivores so that their fitness and survival can be sustained. We used a bioenergetics-based approach to assess whether the energy budget of herbivorous gastropods was promoted when they and their food were reared at the future levels of ocean acidification and warming. If the trophic transfer of energy is sufficient to compensate for the direct negative effects of ocean warming and acidification on herbivores (i.e. energy budget is maintained or enhanced), herbivory is likely maintained in future marine ecosystems. Instead, we found that future climate (i.e. the combination of ocean acidification and warming) induced mass mortality of herbivores as a consequence of energy depletion, despite the greater nutritional quality of their food.

2. Materials and methods

2.1. Experimental protocol

To examine the direct effects of ocean acidification and warming on gastropods, we measured their respiration rate, feeding rate and absorption rate. The indirect effects were observed by measuring the nutritional quality of their food so that the energy budget of gastropods was estimated. If ocean acidification and warming can increase the energy transfer, we hypothesized that (1) the feeding rate and energy budget of gastropods would be elevated under ocean acidification so that their body condition, growth and survival are unaffected or even promoted; (2) the increased energy demand under ocean warming would be insufficiently offset when the elevated temperature is close to the thermal tolerance of gastropods, causing reduced body condition and growth; (3) mortality would be incurred when energy deficit persists, as indicated by reduced body condition. To assess the circumstances where thermal stress is posed on gastropods by ocean warming, we determined the performance of aerobic metabolism along a temperature gradient and critical thermal maximum (CT_{max}) as a proxy for thermal tolerance (Lutterschmidt and Hutchison, 1997).

2.2. Experimental setup

A common subtidal grazing gastropod, *Phasianella australis*, was chosen as the study species due to its abundance in shallow seagrass and rocky reef habitats (Kinloch et al., 2007). This herbivore predominantly feeds on the filamentous algae growing on substratum surface (Keuskamp, 2004), which are called turf algae hereafter (Connell et al., 2014). Individuals were collected in summer from the subtidal zone (~5–10 m depth) at Wirrina Cove, South Australia (35°29′S, 138°15′E). Mesocosm systems were constructed in a constant temperature room (air temperature: 23 °C) to offer a more realistic simulation of the subtidal habitat (Goldenberg et al., 2017). Each mesocosm system was composed of a large circular mesocosm (1.8 m diameter; 1 m depth; ~1800 L) and a small enrichment tank (60 L) where seawater pH and temperature were regulated. Two header tanks (800 L) were made to maintain the inflow of seawater with pre-treatment conditions to the mesocosms (see Supplementary method for the technical details of the mesocosm system). A 2 × 2 crossed design of pCO_2 (400 ppm vs. 1000 ppm) and temperature (21 °C vs. 24 °C) was used with three replicate mesocosms per treatment combination. 21 °C is the average seawater temperature at the collection site in summer, and the elevated pCO_2 and temperature levels simulate the RCP8.5 scenario for the year 2100 (IPCC, 2013). The elevated pCO_2 level was achieved by bubbling the seawater in the header tanks with pure CO_2 and then maintained by aerating the seawater in the enrichment tank with CO_2-enriched air using a gas mixer (Pegas 4000 MF, Columbus Instruments, USA). The desired seawater temperature was maintained using two titanium heaters (300 W × 1 and 500 W × 1), which were put into the enrichment tank. The mesocosm systems were allowed to run for three weeks to ensure that the seawater and habitat conditions were stabilized before experimentation. The seawater carbonate system parameters for the experimental period are shown in Table S1.

Twenty individuals of juvenile *P. australis* (shell length: 10 ± 3.5 mm; total biomass: 4.0 ± 0.25 g) were transferred into each mesocosm and allowed to acclimate for one week prior to commencement of the experiment. Then, they were maintained in the mesocosm for six months. Turf algae growing on the wall of mesocosm were the major food source of *P. australis* in view of their availability and accessibility. Grazing trails cleared of turf algae were observed following consumption by *P. australis*, indicating its non-selective feeding on turf algae. After the 6-month exposure period, mortality was determined by collecting and

enumerating the individuals in each mesocosm ($n = 3$ replicate mesocosms per treatment). An electronic balance was used to measure the total weight of each individual so that the change in total biomass in each mesocosm was determined ($n = 3$ replicate mesocosms per treatment). Following the experiments for energetics (see Section 2.3 below), all individuals were dissected to obtain the flesh weight which was further separated into organ weight and foot weight. The organ weight to flesh weight ratio of each individual was calculated to indicate body condition (Leung et al., 2017b). The initial organ weight to flesh weight ratio was obtained by dissecting additional 20 individuals before the exposure period so that the change in this ratio can be calculated. Body condition is improved when this ratio has positive change, and vice versa. All the weights were measured on a fresh weight basis. To assess the nutritional quality of food, turf algae growing on the wall of mesocosm were collected for the analysis of energy content and C:N ratio using a bomb calorimeter (AC-350, LECO, USA) and CHNS elemental analyser (2400 Series II, Perkin Elmer, USA) ($n = 3$ replicate mesocosms per treatment; 2 trials per replicate), respectively.

2.3. Energetics of gastropods following exposure

Following the 6-month exposure period, energy loss by respiration and energy gain by feeding were estimated because they are the key processes determining energy budget in marine molluscs (Widdows and Johnson, 1988; Wang et al., 2015; Zhang et al., 2015). To measure respiration rate, one individual was transferred into a 73 mL airtight chamber filled with seawater adjusted to the respective pCO_2 and temperature levels of the mesocosm and allowed to rest for 1 h to eliminate potential handling stress. Then, the seawater in the chamber was fully replaced with oxygen-saturated experimental seawater (i.e. pCO_2 and temperature manipulated) with initial dissolved oxygen concentration measured by an optical dissolved oxygen probe (Fibox 4, PreSens, Germany). The chamber was then closed and put into a water bath at the target temperature so that the temperature of seawater in the chamber was maintained. After 1 h, the chamber was opened to measure the final dissolved oxygen concentrations of seawater. Two individuals from each mesocosm were tested and the average respiration rate was taken as a replicate for each mesocosm ($n = 3$ replicate mesocosms per treatment). The background change in dissolved oxygen concentration was corrected by blank samples (i.e. no individual in the chamber). Respiration rate was expressed as oxygen consumed per flesh weight per hour, where the flesh weight was obtained following dissection. The same procedure was also applied before the exposure period (i.e. following the 1-week acclimation period) to examine the temporal change in respiration rate, which can indicate the health status of gastropods after the 6-month exposure period.

Before feeding trials, tiles were put into each mesocosm for one month which allows adequate quantity of turf algae to grow on the tile surface for experimentation. To standardize hunger level, gastropods in each mesocosm were temporarily transferred into a nylon mesh cage without food, which was then put back to the respective mesocosm. After starving for one day, one individual was placed into a clean, tailor-made plastic cage (12 cm × 12 cm × 5 cm), which contained a tile (5 cm × 10 cm) with turf algae growing under the respective treatment conditions. Only the tiles with full coverage of turf algae were selected and used for the feeding trials. Both ends of the cage were covered by nylon mesh to allow continuous water flow. The individual was then put back to the respective mesocosm and allowed to feed in the cage for 3 h. The mesocosm was covered with an opaque tarp so that the feeding trial was carried out in the dark. Two to four individuals from each mesocosm were tested in separate cages and their feeding rates were averaged as a replicate for each mesocosm ($n = 3$ replicate mesocosms per treatment). Each tile was photographed at a fixed distance of 37 cm using a high-definition camera (Canon EOS 1100D) before and after the feeding trial to estimate the total grazed space on the tile (i.e. no turf algae). The photographs were then analysed using software eCognition 9 (Trimble, Germany), which provides high accuracy for measuring spatial coverage. Feeding rate was expressed as the dry weight of turf algae consumed per flesh weight of gastropod per hour, where the dry weight of turf algae per area was measured by scraping the tile with a scalpel to collect the turf algae in a known area, which were then oven-dried and weighed.

To estimate absorption rate (i.e. energy gain by feeding), assimilation efficiency was determined by measuring the organic matter content in turf algae and faeces. The faeces of gastropods in the cages were collected, rinsed with deionized water and dried on a pre-weighed aluminium foil to obtain the dry weight of faeces. The ash-free dry weights of turf algae and faeces were determined by weight loss on ignition at 550 °C in a muffle furnace for 6 h so that assimilation efficiency was calculated using Conover ratio (Conover, 1966):

$$AE(\%) = \frac{F' - E'}{(1 - E')(F')} \times 100$$

where AE is the assimilation efficiency; F' is the ash-free dry weight to dry weight ratio of food; E' is the ash-free dry weight to dry weight ratio of faeces.

Absorption rate can then be calculated by multiplying the feeding rate by assimilation efficiency, whereas energy budget is given by energy gain by feeding minus energy loss by respiration. The energy content of turf algae and a conversion factor of 14.14 J mg O_2^{-1} were used to convert feeding rate and respiration rate into energy equivalent (Elliott and Davison, 1975), respectively.

2.4. Thermal tolerance and aerobic metabolism along a temperature gradient

Aerobic metabolism of gastropods was assessed by measuring respiration rate from 15 °C to 35 °C with an increasing temperature ramp of 2 °C h^{-1} (Giomi et al., 2016), whereas thermal tolerance was indicated by CT_{max} at which motor coordination is lost (Lutterschmidt and Hutchison, 1997). The procedure for this experiment has been previously described in Leung et al. (2017a) (see Supplementary method for the details).

2.5. Statistical analysis

Two-way permutational analysis of variance with pCO_2 and temperature as fixed factors was applied to test their effects on the aforementioned response variables (i.e. calorific value, C:N ratio, total biomass, mortality and respiration rate) with each mesocosm as a replicate, except for organ weight to flesh weight ratio with each individual as a replicate in view of the large number of individuals used. Given the substantial mortality under combined elevated pCO_2 and temperature conditions, feeding trials were not performed for this treatment. Therefore, t-test was applied to examine the effects of pCO_2 and temperature on feeding rate and energy budget.

3. Results

The energy content of turf algae was promoted from 9.8 kJ g^{-1} (control) to 15.0 kJ g^{-1} by elevated temperature and 13.9 kJ g^{-1} by combined elevated pCO_2 and temperature (Fig. 1a, Table S2). However, pCO_2 in isolation had no detectable effect. C:N ratio was reduced by elevated pCO_2 at ambient temperature, meaning that CO_2 enrichment can increase the relative nitrogen content in turf algae (Fig. 1b, Table S2). Elevated temperature also reduced the C:N ratio of turf algae compared to the control, but this positive effect of nitrogen enrichment was slightly reduced when combined with elevated pCO_2.

The organ weight to flesh weight ratio of *P. australis* increased by ~7% under control and ~5% under elevated pCO_2 conditions, but remained nearly unchanged under elevated temperature in isolation (Fig. 2a).

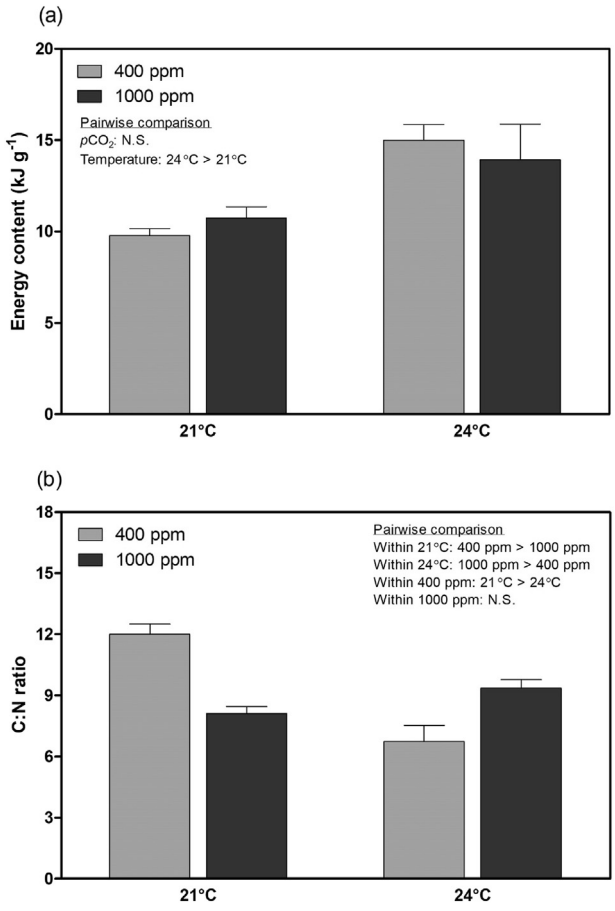

Fig. 1. Energy content and C:N ratio of turf algae growing at different pCO_2 and temperature levels (mean ± S.E., $n = 3$). N.S.: Not significant.

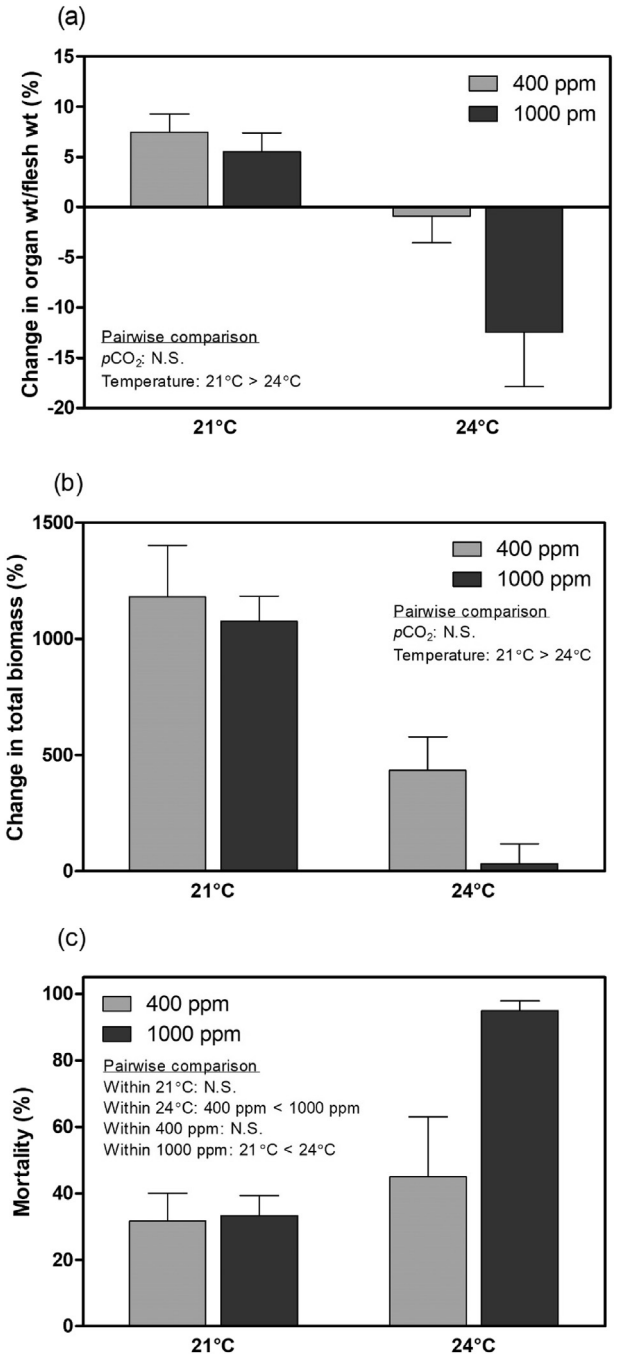

Fig. 2. (a) Change in organ weight to flesh weight ratio (mean + S.E.; $n = 41$ individuals for 21 °C, 400 ppm, $n = 40$ individuals for 21 °C, 1000 ppm, $n = 32$ individuals for 24 °C, 400 ppm and $n = 3$ individuals for 24 °C, 1000 ppm), (b) change in total biomass and (c) mortality of *P. australis* following the 6-month exposure period at different pCO_2 and temperature levels (mean + S.E.; $n = 3$ replicate mesocosms). N.S.: Not significant.

Statistically, this ratio was reduced by elevated temperature, whilst the effect of pCO_2 was not discernible (Table S2). Yet, this ratio decreased markedly by ~12% under combined elevated pCO_2 and temperature conditions, indicating reduced energy reserves and body condition. Elevated temperature, but not elevated pCO_2, negatively impacted the increase in total biomass of *P. australis* in the mesocosms (Fig. 2b, Table S2). Neither elevated pCO_2 nor temperature in isolation significantly affected the mortality of *P. australis* (c.f. control), ranging from 33% to 45%. However, mortality increased drastically to ~90% under combined elevated pCO_2 and temperature conditions (Fig. 2c, Table S2).

Compared to the control, the feeding rate of *P. australis* increased by approximately two times at elevated pCO_2 ($t = 3.81$, $p = 0.019$), but remained insignificantly changed at elevated temperature ($t = 1.58$, $p = 0.189$) following the 6-month exposure period (Fig. 3a). Assimilation efficiency, ranging from 60% to 73%, was neither affected by elevated pCO_2 ($t = 0.96$, $p = 0.391$) nor temperature ($t = 2.75$, $p = 0.051$) (Fig. 3b). Compared to the control, energy budget was about three-fold higher at elevated pCO_2 ($t = 4.59$, $p = 0.010$) and two-fold higher at elevated temperature ($t = 3.37$, $p = 0.028$) (Fig. 3c). A significant negative correlation was found between C:N ratio and feeding rate (Fig. 3d), meaning that the feeding rate of gastropods had a positive relationship with the relative nitrogen content of their food.

After the 6-month exposure period, the respiration rate of *P. australis* was substantially reduced by nearly 40% under combined elevated pCO_2 and temperature conditions (Fig. 4, Table S2). Yet, elevated pCO_2 or temperature in isolation had limited effect on the change in respiration rate (Fig. S1 for the respiration rate before and after exposure).

Along an increasing temperature ramp, *P. australis* was inactive below 17 °C but became more active with increasing temperature from 17 °C to 25 °C (Fig. 5). Motor coordination was lost at 27 °C which represents the CT_{max} (i.e. upper thermal tolerance) of *P. australis*. Respiration rate increased with temperature from 15 °C to 25 °C (maximum), above which the respiration rate declined gradually with increasing temperature irrespective of pCO_2 level (Fig. 5). The effect of pCO_2 on respiration rate was not discernible.

4. Discussion

Enhanced trophic transfer from primary producers to herbivores may be the key mechanism allowing herbivores to maintain their populations under ocean acidification and warming (Boyd and Brown,

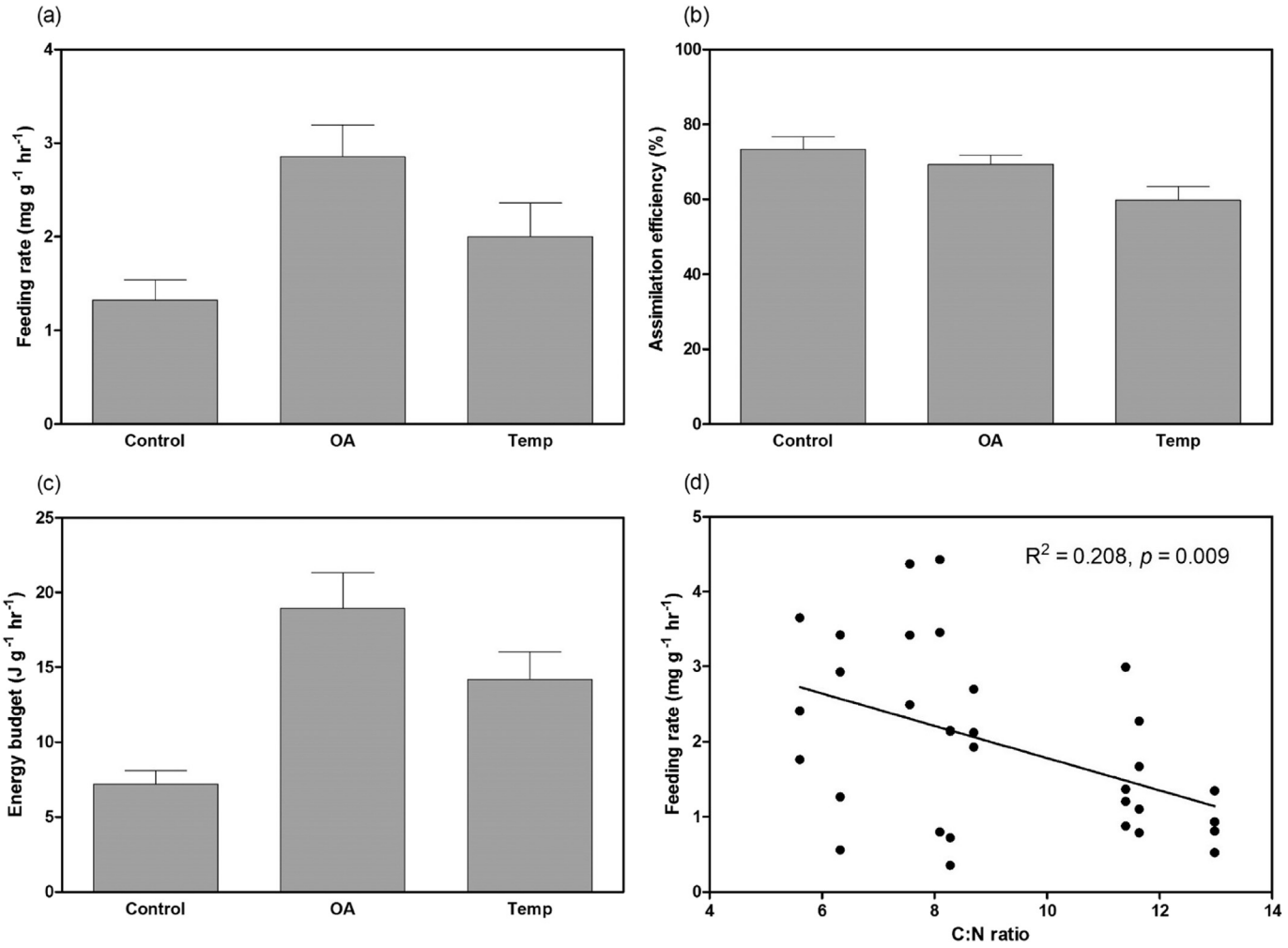

Fig. 3. (a) Feeding rate, (b) assimilation efficiency and (c) energy budget of *P. australis* under different treatment conditions (mean + S.E.; $n = 3$); (d) Relationship between feeding rate of *P. australis* and C:N ratio of turf algae. Control: 21 °C, 400 ppm; OA: 21 °C, 1000 ppm; Temp: 24 °C, 400 ppm.

2015; Rosenblatt and Schmitz, 2016). Here, we demonstrated that the boosting effect through trophic transfer elevates the energy budget of a common marine herbivore under ocean acidification or warming in isolation, which is favourable for long-term survival. However, such indirect positive effect was overwhelmed by the direct negative effect of these climate changes stressors in combination, leading to a substantial decline in fitness and survival.

It has been well-recognized that ocean acidification disrupts the energy homeostasis of marine organisms, especially calcifying molluscs, because they require extra metabolic energy for acid-base regulation and calcification (Pörtner, 2008; Thomsen et al., 2015). In addition, reduced pH can undermine their feeding performance, energy gain and eventually growth (Fabry et al., 2008; Gazeau et al., 2013; Leung et al., 2015). Nevertheless, we found that ocean acidification has little

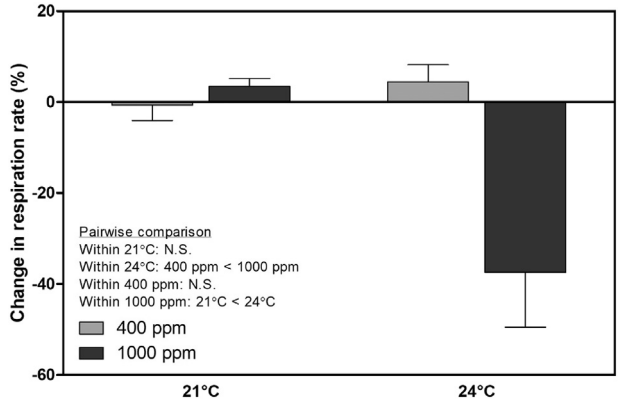

Fig. 4. Change in respiration rate of *P. australis* following the 6-month exposure period at different pCO_2 and temperature levels (mean + S.E.; $n = 3$). N.S.: Not significant.

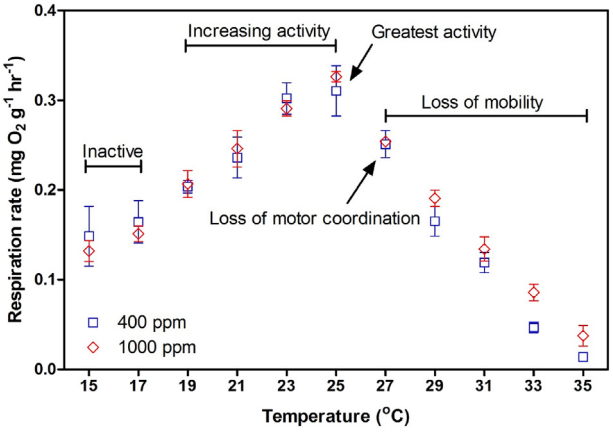

Fig. 5. Respiration rate of *P. australis* along an increasing temperature ramp from 15 °C to 35 °C at different pCO_2 levels (mean ± S.E.; $n = 3$).

influence on the body condition, biomass and survival of *P. australis*, suggesting that energy homeostasis was maintained. In other words, the elevated energy demand under ocean acidification was probably offset by the increased feeding rate and hence energy gain. Based on the metabolic theory of ecology (Brown et al., 2004), increased feeding rate can be driven by elevated metabolic rate, as shown in some grazing gastropods (Mertens et al., 2015; McSkimming et al., 2016), but the respiration rate of our study gastropod was not upregulated by ocean acidification (Fig. S1), meaning that the feeding rate is not driven by metabolic rate. We suggest that the increased feeding rate under ocean acidification is mediated by the reduced C:N ratio of turf algae, which could be attributed to the higher assimilation of nitrogen or more efficient photosynthesis that allows reallocation of nitrogen in metabolic processes (Zou, 2005; Hurd et al., 2009; Koch et al., 2013; Xu et al., 2017). As nitrogen is often limiting for herbivores (Mattson, 1980), the higher relative nitrogen content in turf algae can enhance the feeding rate of gastropods, as also shown in other herbivores (Falkenberg et al., 2013; Ghedini and Connell, 2016; Kamya et al., 2017). Regardless of the underlying mechanism, we showed that the increased feeding rate on the more nutritious turf algae promotes energy budget, ultimately enabling the gastropods to maintain fitness under ocean acidification. Indeed, such boosting effect through trophic transfer could outweigh the direct negative effects of ocean acidification (Falkenberg et al., 2013; Kamya et al., 2017), and even boost the population of herbivores as observed under natural (Connell et al., 2017; Vizzini et al., 2017) and mesocosm conditions (Goldenberg et al., 2017).

Ocean warming in isolation was also shown to enhance the energy budget of gastropods, but their body condition and biomass were lower than those in the control, suggesting that less energy was allocated to growth. Thermal tolerance is often the overriding factor determining the effect of elevated temperature on aerobic scope (or energy budget) and hence growth. We found that the CT_{max} of *P. australis* appears at 27 °C where motor coordination is lost and respiration rate is declining. As such, aerobic scope and overall performance are diminished according to the oxygen- and capacity-limited thermal tolerance concept (Pörtner, 2002, 2012). To prevent this acute thermal stress, locomotory activity was maximized at 25 °C as the avoidance behaviour. Since the respiration rate at elevated temperature (i.e. 24 °C) was close to the maximum at 25 °C, the gastropods likely suffered from sublethal thermal stress in the mesocosms, which can reduce energy budget and growth in the long term (Pörtner, 2002, 2012; Sokolova, 2013; Gianguzza et al., 2014; Leung et al., 2017a). To compensate for the greater energy demand at elevated temperature, energy gain by feeding should be promoted, but our study gastropod failed to increase feeding rate possibly due to the sublethal thermal stress (Leung et al., 2017a). Nevertheless, the higher energy content of turf algae increases energy gain per feeding effort and hence energy budget, which helps offset the elevated energy demand under ocean warming. Despite the advantage of this boosting effect, growth was still retarded, implying that the energy budget of gastropods is allocated mostly to somatic maintenance and less to growth and reproduction based on the dynamic energy budget model (Nisbet et al., 2012). Indeed, survival under sublethal thermal stress requires a disproportionate energy allocation for somatic maintenance because cellular and structural damage can already be inflicted (Pörtner, 2002; Somero, 2002). For example, production of heat shock proteins, which incurs substantial energy demand, is upregulated under sublethal thermal stress to repair damaged proteins and stabilize denaturing proteins (Miller et al., 2009; Tomanek, 2010). Consequently, the boosting effect via trophic transfer was inadequate to fully compensate for the direct negative effects of sublethal thermal stress on energy budget, resulting in reduced body condition.

The adverse effects of elevated temperature were exacerbated when combined with elevated pCO_2, as indicated by the mass mortality of gastropods. This suggests that the boosting effect through trophic transfer was outweighed by the synergistic effect of ocean acidification and warming. Under stressful conditions (e.g. sublethal thermal stress), aerobic metabolism should be promoted to provide sufficient metabolic energy for molecular defence mechanisms, such as enhanced production of heat shock proteins and antioxidative enzymes (Pörtner, 2002; Somero, 2002; Tomanek, 2010). However, the aerobic metabolism of gastropods dropped substantially under combined elevated pCO_2 and temperature conditions (c.f. elevated temperature in isolation), implying not only insufficient metabolic energy for basal maintenance, but also impairment in cellular functions (e.g. energy production by mitochondria) (Pörtner, 2002; Sokolova et al., 2012). The considerable reduction in metabolic energy would worsen overall biological performance (Pörtner, 2012; Sokolova, 2013), including feeding (Mertens et al., 2015). Although we did not examine feeding rate due to the mass mortality, energy reserves (i.e. organ weight to flesh weight ratio) were markedly reduced, indicating a critical mismatch between energy gain and energy demand. Using energy reserves only provides temporary relief under adverse environmental conditions and is maladaptive in the long term (Sokolova, 2013). When energy reserves are depleted, vital cellular functions and physiological processes will be arrested, culminating in mortality and thus affecting population persistence (Leung et al., 2017a). We propose that this energetics-based mechanism is fundamental to accounting for the combined negative effects of ocean acidification and warming on the fitness and survival of marine organisms, as observed in corals (Prada et al., 2017), bivalves (Talmage and Gobler, 2011; Ivanina et al., 2013) and gastropods (Melatunan et al., 2013).

Herbivory is a fundamental component of ecosystem stability because it not only determines the flux of energy and materials from primary producers to herbivores, but also has the capacity to compensate for climate-driven changes in primary production (Post and Pedersen, 2008; Ghedini et al., 2015). Indeed, the stability of food webs often reflects such consumer-resource interactions (May, 1973; McCann et al., 1998) and their capacity to adjust to environmental changes (Connell and Ghedini, 2015). We demonstrated that future climate (i.e. ocean acidification and warming) can increase the nutritional quality of primary producers to boost the energy budget of herbivores. However, we found that this positive effect on food quality could be overwhelmed by the elevated energy demand of ocean acidification and warming on the herbivores themselves, leading to reduced fitness and survival. In such situations, the loss of trophic compensation may increase the chance of ecosystem collapse (e.g. climate-driven collapse of kelp forests, Connell et al., 2018). Whilst herbivores could maintain their homeostasis under increasingly stressful conditions (Ghedini and Connell, 2016), further increase in stress inevitably cross a tipping point at which the increasing metabolic costs cause this compensatory mechanism to fail. As such, the innate capacity of herbivores to control their blooming resources fails and runaway primary production is likely to ensue.

Acknowledgements

Support was provided by the IPRS Scholarship from the University of Adelaide to J.Y.S.L., an Australian Research Council Discovery Program grant to B.D.R. and S.D.C. (DP150104263), and ARC Future Fellowships to S.D.C. (FT0991953) and I.N. (FT120100183).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.05.161.

References

Boyd, P.W., Brown, C.J., 2015. Modes of interactions between environmental drivers and marine biota. Front. Mar. Sci. 2, 9.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.
Connell, S.D., Ghedini, G., 2015. Resisting regime-shifts: the stabilising effect of compensatory processes. Trends Ecol. Evol. 30, 513–515.

Connell, S.D., Foster, M.S., Airoldi, L., 2014. What are algal turfs? Towards a better description of turfs. Mar. Ecol. Prog. Ser. 495, 299–307.
Connell, S.D., Doubleday, Z.A., Hamlyn, S.B., Foster, N.R., Harley, C.D.G., Helmuth, B., Kelaher, B.P., Nagelkerken, I., Sarà, G., Russell, B.D., 2017. How ocean acidification can benefit calcifiers. Curr. Biol. 27, R95–R96.
Connell, S.D., Doubleday, Z.A., Foster, N.R., Hamlyn, S.B., Harley, C.D.G., Helmuth, B., Kelaher, B., Nagelkerken, I., Rodgers, K.L., Sarà, G., Russell, B.D., 2018. The duality of ocean acidification as a resource and a stressor. Ecology 99, 1005–1010.
Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11, 338–345.
Cruz-Rivera, E., Hay, M.E., 2001. Effects of macroalgal traits on the feeding and fitness of an herbivorous amphipod: the relative importance of selectivity, mixing, and compensation. Mar. Ecol. Prog. Ser. 218, 249–266.
Elliott, J.M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19, 195–201.
Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J. Mar. Sci. 65, 414–432.
Falkenberg, L.J., Russell, B.D., Connell, S.D., 2013. Future herbivory: the indirect effects of enriched CO_2 may rival its direct effects. Mar. Ecol. Prog. Ser. 492, 85–95.
Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J.P., O'Connor, W.A., Martin, S., Pörtner, H.O., Ross, P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. Mar. Biol. 160, 2207–2245.
Ghedini, G., Connell, S.D., 2016. Organismal homeostasis buffers the effects of abiotic change on community dynamics. Ecology 97, 2671–2679.
Ghedini, G., Russell, B.D., Connell, S.D., 2015. Trophic compensation reinforces resistance: herbivory absorbs the increasing effects of multiple disturbances. Ecol. Lett. 18, 182–187.
Gianguzza, P., Visconti, G., Gianguzza, F., Vizzini, S., Sarà, G., Dupont, S., 2014. Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula* early life stages to CO_2-driven acidification. Mar. Environ. Res. 93, 70–77.
Giomi, F., Mandaglio, C., Ganmanee, M., Han, G.D., Dong, Y.W., Williams, G.A., Sarà, G., 2016. The importance of thermal history: costs and benefits of heat exposure in a tropical, rocky shore oyster. J. Exp. Biol. 219, 686–694.
Goldenberg, S.U., Nagelkerken, I., Ferreira, C.M., Ullah, H., Connell, S.D., 2017. Boosted food web productivity through ocean acidification collapses under warming. Glob. Chang. Biol. 23, 4177–4184.
Goldenberg, S.U., Nagelkerken, I., Marangon, E., Bonnet, A., Ferreira, C.M., Connell, S.D., 2018. Ecological complexity buffers the impacts of future climate on marine consumers. Nat. Clim. Chang. 8, 229–233.
Gordillo, F.J.L., Carmona, R., Viñegla, B., Wiencke, C., Jiménez, C., 2016. Effects of simultaneous increase in temperature and ocean acidification on biochemical composition and photosynthetic performance of common macroalgae from Kongsfjorden (Svalbard). Polar Biol. 39, 1993–2007.
Heldt, K.A., Connell, S.D., Anderson, K., Russell, B.D., Munguia, P., 2016. Future climate stimulates population out-breaks by relaxing constraints on reproduction. Sci. Rep. 6, 33383.
Hemmi, A., Jormalainen, V., 2002. Nutrient enhancement increases performance of a marine herbivore via quality of its food alga. Ecology 83, 1052–1064.
Hurd, C.L., Hepburn, C.D., Currie, K.I., Raven, J.A., Hunter, K.A., 2009. Testing the effects of ocean acidification on algal metabolism: considerations for experimental designs. J. Phycol. 45, 1236–1251.
IPCC, 2013. In: Stocker, T.F., et al. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Ivanina, A.V., Dickinson, G.H., Matoo, O.B., Bagwe, R., Dickinson, A., Beniash, E., Sokolova, I. M., 2013. Interactive effects of elevated temperature and CO_2 levels on energy metabolism and biomineralization of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166, 101–111.
Kamya, P.Z., Byrne, M., Mos, B., Hall, L., Dworjanyn, S.A., 2017. Indirect effects of ocean acidification drive feeding and growth of juvenile crown-of-thorns starfish, *Acanthaster planci*. Proc. R. Soc. B 284, 20170778.
Keuskamp, D., 2004. Limited effects of grazer exclusion on the epiphytes of *Posidonia sinuosa* in South Australia. Aquat. Bot. 78, 3–14.
Kinloch, M.A., Brock, D.J., Kirkman, H., Laperousaz, T., 2007. Seagrass Biodiversity on Kangaroo Island. KI NRM Board Coast and Marine Program Report No. CMP07/004.
Koch, M., Bowes, G., Ross, C., Zhang, X.H., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob. Chang. Biol. 19, 103–132.
Leung, J.Y.S., Russell, B.D., Connell, S.D., Ng, J.C.Y., Lo, M.M.Y., 2015. Acid dulls the senses: impaired locomotion and foraging performance in a marine mollusk. Anim. Behav. 106, 223–229.
Leung, J.Y.S., Connell, S.D., Russell, B.D., 2017a. Heatwaves diminish the survival of a subtidal gastropod through reduction in energy budget and depletion of energy reserves. Sci. Rep. 7, 17688.
Leung, J.Y.S., Russell, B.D., Connell, S.D., 2017b. Mineralogical plasticity acts as a compensatory mechanism to the impacts of ocean acidification. Environ. Sci. Technol. 51, 2652–2659.
Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561–1574.
Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11, 119–161.
May, R.M., 1973. Qualitative stability in model ecosystems. Ecology 54, 638–641.
McCann, K.S., Hastins, A., Strong, D.R., 1998. Trophic cascades and trophic trickles in pelagic food webs. Proc. R. Soc. B 265, 205–209.
McSkimming, C., Russell, B.D., Tanner, J.E., Connell, S.D., 2016. A test of metabolic and consumptive responses to local and global perturbations: enhanced resources stimulate herbivores to counter expansion of weedy species. Mar. Freshw. Res. 67, 96–102.
Melatunan, S., Calosi, P., Rundle, S.D., Widdicombe, S., Moody, A.J., 2013. Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. Mar. Ecol. Prog. Ser. 472, 155–168.
Mertens, N.L., Russell, B.D., Connell, S.D., 2015. Escaping herbivory: ocean warming as a refuge for primary producers where consumer metabolism and consumption cannot pursue. Oecologia 179, 1223–1229.
Miller, L.P., Harley, C.D.G., Denny, M.W., 2009. The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea*. Funct. Ecol. 23, 756–767.
Nagelkerken, I., Connell, S.D., 2015. Global alteration of ocean ecosystem, functioning due to increasing human CO_2 emissions. Proc. Natl. Acad. Sci. U. S. A. 112, 13272–13277.
Nisbet, R.M., Jusup, M., Klanjscek, T., Pecquerie, L., 2012. Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. J. Exp. Biol. 215, 892–902.
Pörtner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 132, 739–761.
Pörtner, H.O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar. Ecol. Prog. Ser. 373, 203–217.
Pörtner, H.O., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar. Ecol. Prog. Ser. 470, 273–290.
Post, E., Pedersen, C., 2008. Opposing plant community responses to warming with and without herbivores. Proc. Natl. Acad. Sci. U. S. A. 105, 12353–12358.
Prada, F., Caroselli, E., Mengoli, S., Brizi, L., Fantazzini, P., Capaccioni, B., Pasquini, L., Fabricius, K.E., Dubinsky, Z., Falini, G., Goffredo, S., 2017. Ocean warming and acidification synergistically increase coral mortality. Sci. Rep. 7, 40842.
Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195–214.
Rosenblatt, A.E., Schmitz, O.J., 2016. Climate change, nutrition, and bottom-up and top-down food web processes. Trends Ecol. Evol. 31, 965–975.
Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. 53, 597–608.
Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15.
Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr. Comp. Biol. 42, 780–789.
Talmage, S.C., Gobler, C.J., 2011. Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of Northwest Atlantic bivalves. PLoS ONE 6, e26941.
Thomsen, J., Haynert, K., Wegner, K.M., Melzner, F., 2015. Impact of seawater carbonate chemistry on the calcification of marine bivalves. Biogeosciences 12, 4209–4220.
Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. 213, 971–979.
Vizzini, S., Martínez-Crego, B., Andolina, C., Massa-Gallucci, A., Connell, S.D., Gambi, M.C., 2017. Ocean acidification as a driver of community simplification via the collapse of higher-order and rise of lower-order consumers. Sci. Rep. 7, 4018.
Wang, Y., Li, L., Hu, M., Lu, W., 2015. Physiological energetics of the thick shell mussel *Mytilus coruscus* exposed to seawater acidification and thermal stress. Sci. Total Environ. 514, 261–272.
Widdows, J., Johnson, D., 1988. Physiological energetics of *Mytilus edulis*: scope for growth. Mar. Ecol. Prog. Ser. 46, 113–121.
Xu, Z., Gao, G., Xu, J., Wu, H., 2017. Physiological response of a golden tide alga (*Sargassum muticum*) to the interaction of ocean acidification and phosphorus enrichment. Biogeosciences 14, 671–681.
Zhang, H., Shin, P.K.S., Cheung, S.G., 2015. Physiological responses and scope for growth upon medium-term exposure to the combined effects of ocean acidification and temperature in a subtidal scavenger *Nassarius conoidalis*. Mar. Environ. Res. 106, 51–60.
Zou, D., 2005. Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed *Hizikia fusiforme* (Sargassaceae, Phaeophyta). Aquaculture 250, 726–735.